MITOCHONDRIAL DNA PATHOGENIC MUTATIONS IN MULTIPLE SYMMETRIC LIPOMATOSIS

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SHORT REPORT

MITOCHONDRIAL DNA PATHOGENIC MUTATIONS IN MULTIPLE SYMMETRIC LIPOMATOSIS

SHORT RUNNING TITLE: A new mtDNA mutation causing MSL

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available upon request.

ABSTRACT

The frequency of dermatological manifestations in diseases due to mitochondrial DNA mutations is not well known, although multiple symmetric lipomatosis has been repeatedly associated to mitochondrial DNA mutations. Here, we present a patient suffering from multiple symmetric lipomatosis and other skin signs. We found a new mitochondrial DNA mutation, m.8357T>C, in the $tRNA^{Lys}$-coding gene and, using a cybrid approach, confirmed its pathogenicity. A meta-analysis of the dermatological signs of the patient shows that they are not common in patients with confirmed mitochondrial DNA mutations and suggests that, in these cases, lipomatosis is not related to the oxidative phosphorylation dysfunction, but to an alteration of an additional function associated to particular mitochondrial tRNAs.

KEYWORDS

Mitochondrial DNA mutation; multiple symmetric lipomatosis; erythema; hyperkeratosis; dermatologic manifestations.

INTRODUCTION

Several key components of the oxidative phosphorylation (OXPHOS) system, the main biochemical pathway for energy production, are coded in the mitochondrial DNA (mtDNA). In OXPHOS disorders due to mtDNA pathological mutations, the most affected tissues are usually those with the highest energy requirements. Cultured dermal fibroblasts are frequently used for diagnosing mitochondrial diseases due to mtDNA mutations, because OXPHOS defects are detectable in these cells [1]. However, OXPHOS disorders are not normally accompanied by obvious skin manifestations [2].

Here, we describe a patient with multiple symmetric lipomatosis (MSL), a rare disease primarily involving adipose tissue and characterized by the presence of not encapsulated fat masses, symmetrically disposed at characteristic body sites (neck, trunk, proximal parts of upper and lower limbs). The patient also showed other skin signs. We found a novel mutation in his mtDNA and confirmed its pathogenicity. Then, we review the literature on dermatologic manifestations in patients with confirmed mtDNA pathological mutations.

MATERIALS AND METHODS

DNA extraction, mtDNA sequencing, nuclear DNA (nDNA) genetic fingerprint, cybrids construction and analyses were performed as previously reported [3,4].

RESULTS

Case report

We described previously the case of a 37-year-old male suffering from MSL (Figure 1A, B) [5]. At the age of 9-year-old, he presented with erythematous-scaly and pruritic plaques on the face, forearms and the palms of both hands. Lesions on the palms were repeated at 17 and 37-year-old. The skin was dry with hyperkeratosis on both hands with predominance of palms. Now, at the age of 47-year-old, desquamative lesions in right thumb and index are appreciated (Figure 1C). The patient (III-1 in Figure 1D) also showed brachycephaly and facial asymmetry, degenerative discopathy, bilateral microlithiasis, sporadic elevation of blood pressure, hyperuricemia and mixed dyslipidemia. No neurological abnormalities or important developmental problems were reported.

The mother of the proband (II-1 in Figure 1D) presented with lipoma at the abdominal level (16 x 12 cm) and in the left shoulder (9 x 7 cm). She also suffered from osteoarthritis at the cervical level, a herniated disc at L4-L5 and controlled arterial hypertension. A maternal half sister (III-2) presented lipoma type lesions at the abdominal level and the maternal half brother (III-3), from a third father, was healthy. Four paternal half sibs from two different mothers were also healthy (Figure 1D).

Ethical approval for this study was obtained from involved Institutional Review Boards. Written informed consent from the patient was also obtained.

mtDNA analysis

mtDNA sequencing showed an m.8357T>C heteroplasmic transition in the *MT-TK* gene coding for the $tRNA^{Lys}$ (Figure 2A). The mutation percentage was 53 and 65 % in two different blood samples from the patient (III-1 in Figure 1D), and 43 and 38 % in blood samples of his mother (II-1) and half maternal sister (III-2), respectively (Figure 2B). This

mutation has not been previously found in 49,135 human mtDNA sequences (GenBank, accessed October 31st 2019). The change breaks a Watson-Crick base pair in the $tRNA^{Lys}$ acceptor stem (Figure 2C), which is found in 112 out of 114 mammal species (http://mamit-tRNA.u-strasbg.fr/). According to MitoTIP [6], an *in silico* tool for predicting pathogenicity of novel mtDNA tRNA variants, m.8357T>C would be possibly pathologic.

Confirmation of pathogenicity by the use of cybrid cell lines

To confirm the pathogenicity of the mutation, we generated cybrids by fusion of osteosarcoma-143B cells with no mtDNA (rho^0 cells) and platelets from the patient or controls. Since platelets contain mitochondria and mtDNA but not nucleus or nDNA, the fusion allows the transfer of the mtDNA to a common nuclear background. The resulting cybrid cell lines share their nDNA but differ in their mtDNA. Therefore, phenotypic differences among them will be due to the mtDNA that they harbor. We built three different cybrids: one with the m.8357T>C mutation (O8357) of the patient; one positive control harboring an already confirmed $tRNA^{lys}$ pathological mutation, m.8344A>G (O8344); and one negative control with no pathological mutations (Owt).

First of all, we confirmed that all the three cybrids had the same nDNA. Fifteen out of 16 short tandem repeats (STRs) were shared in these cybrids (including 8 out of the 9 STR markers reported in the American Type Culture Collection for the osteosarcoma-143B cell line), thus confirming the nDNA homogeneity. Owt and O8344 cybrids had gained a new allele for the CSF1PO marker (12 and 13 versus only 12). We next confirmed the mtDNA genetic background in the cybrids (GenBank MN095205, MN095206, and MN095207 for Owt, O8344 and O8357, respectively). The O8357 cybrid was homoplasmic for the m.8357T>C mutation and the O8344 was heteroplasmic (55 %) for the m.8344A>G mutation (Figure 3A).

The O8357 cybrid showed reduced growth rate, both in glucose and galactose media, when compared with the Owt cybrid (Figure 3B). Moreover, like for the O8344 cybrid, the endogenous, leak and uncoupled oxygen consumption were significantly lower in O8357 cells (Figure 3C). The specific activity and quantity of respiratory complex IV (CIV), and p.MT-CO1 subunit levels from CIV were also significantly reduced in both cybrids when compared with the control cybrid (Figure 3D-F).

Literature meta-analysis

A review of the literature searching for the skin manifestations found in our patient, allowed us to find 49 index cases, plus 61 maternal relatives, harboring mtDNA pathological mutation and suffering from MSL (Supplementary Table): 16.3 % had mtDNA deletions; 4.1 % had point mutations in $tRNA^{Leu}$ and 79.6 % had point mutations in $tRNA^{Lys}$.

Our patient also showed hands hyperkeratosis mainly in the palms. This sign was also found in four pedigrees, harboring the m.7445A>G mutation that affects the processing of $tRNA^{Ser}$ showing hyperkeratosis as a sign of a keratoderma palmoplantar and deafness syndrome [7-10]. Hyperkeratosis was also described in a patient with MELAS syndrome harboring an m.3243A>G mutation in the $tRNA^{Leu}$ [11]. Regarding the erythematous-scaly and pruritic plaques, we only found two previous patients with mtDNA pathological mutations and showing these plaques. Both patients harbored the m.3243A>G mutation in the $tRNA^{Leu}$ [11,12].

DISCUSSION

Our results strongly support that the new maternally transmitted m.8357T>C transition is the etiologic factor for the MSL of this patient. This is sustained by the fact that this change was not previously found in a large collection of mtDNA sequences, it was heteroplasmic and broke a conserved Watson-Crick base pair in the base of the $tRNA^{Lys}$ acceptor stem. Moreover, when the patient's mtDNA was transferred to a rho^0 cell line, the OXPHOS defect was also transmitted to the cybrids, in a similar way to other already confirmed $tRNA^{Lys}$ pathological mutations [13,14]. Finally, lipomas segregated in the pedigree along with the mutation.

The frequency of skin abnormalities in mtDNA disorders is not known [15], possibly because they are not generally reported in the description of these pathologies [2]. Interestingly, two associations between mtDNA mutations and skin manifestations have been repeatedly described: m.7445A>G mutation and keratoderma palmoplantar; and mutations in the *MT-TK* gene and MSL. However, our meta-analysis and other studies suggest that skin anomalies are not frequent in patients with confirmed mtDNA mutations [16].

Thus, MSL could not due to the OXPHOS dysfunction originated by a defect in the mitochondrial translation, but to the alteration of an additional function associated to these particular tRNAs [17]. In this sense, it has been suggested that MSL could be the result of a disorder of proliferation and differentiation of brown adipose tissue (BAT) cells [18]. The BAT-specific *UCP1* mRNA is expressed in lipomas from MSL patients with the m.8344A>G mutation [19]. This mutation is associated to increased mitophagy [20] that promotes the release of mitochondrial $tRNA^{Lys}$ molecules. Once in the cytoplasm $tRNA^{Lys}$ interacts with the cytosolic YBX1 protein [21]. YBX1 cooperates with EWS protein and both activate the transcription of Bmp7 [22], a critical early brown adipogenic factor. Since not all patients harboring $tRNA^{Lys}$ mutations develop lipomas, another factor must be also involved in the process.

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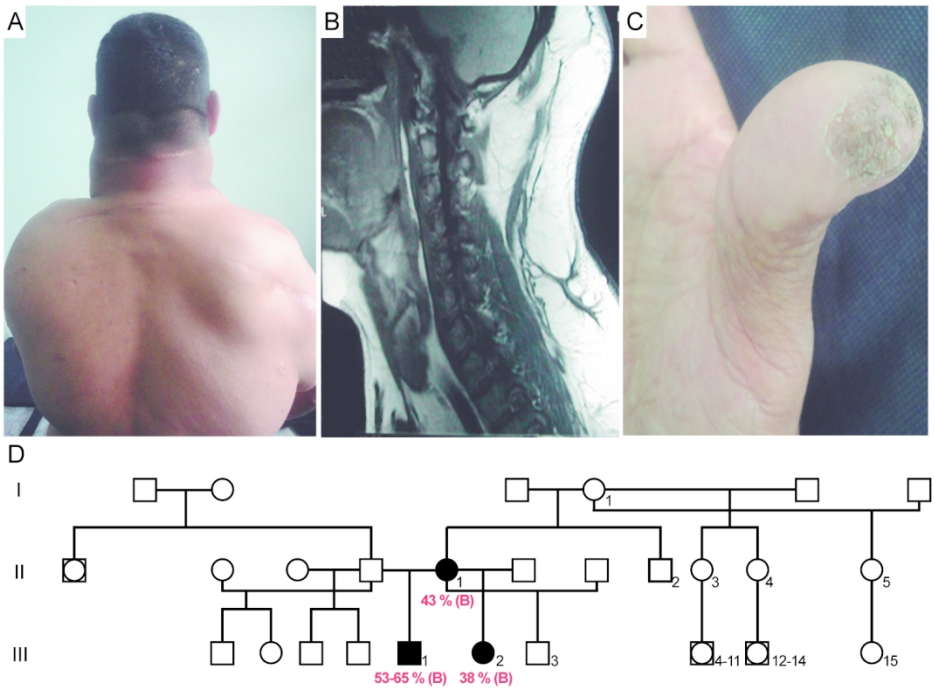
FIGURE LEGENDS

Figure 1. Clinical characteristics of the patient. A) Posterior view of the neck and shoulders with increased volume due to lipomas. B) Computational tomography of the neck (sagittal section) showing a space-occupying lesion hypodense with well-defined edges in relation to fat content. C) Desquamative, dirty looking lesion on right thumb. D) Pedigree. III-1 is the proband. The mutation percentage in blood (B) is indicated.

Figure 2. m.8357T>C mutation in the mtDNA. A) Electropherogram of a segment of the patient's mtDNA sequence showing the heteroplasmic m.8357T>C transition. B) RFLP gel showing the mutation load in blood from the patient (P1 and P2), the mother (M) and the half-sister (HS). A, amplicon. C-, negative control. C) Secondary structure of the mitochondrial $tRNA^{lys}$. The mutated position is indicated.

Figure 3. Biochemical characterization of the cybrid cell lines. A) RFLP gel confirming the presence of the mtDNA pathological mutations m.8344A>G and m.8357T>C in the respective cell line. M, molecular weight marker. A, amplicon. B) Growing curves in glucose or galactose media. C) Oxygen consumption. E, endogenous respiration; L, leaking respiration; and U, uncoupled respiration. White, grey and black bars represent Owt, O8344 and O8357 cybrids, respectively. *, $p < 0.05$ (vs Owt). D) Respiratory complex IV (CIV) specific activity (s.a.) normalized by citrate synthase (CS) s.a. E) CIV quantity (q.) normalized by CS s.a. F) Western blot showing the amount of the mtDNA-encoded p.MT-CO1 subunit from CIV. The levels of the nDNA-encoded SDHA subunit from respiratory complex II (CII) have been used to normalize.



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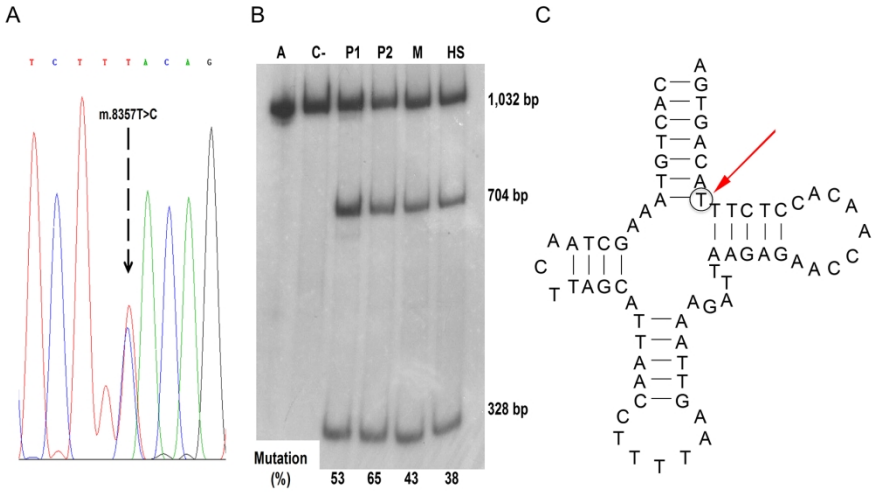

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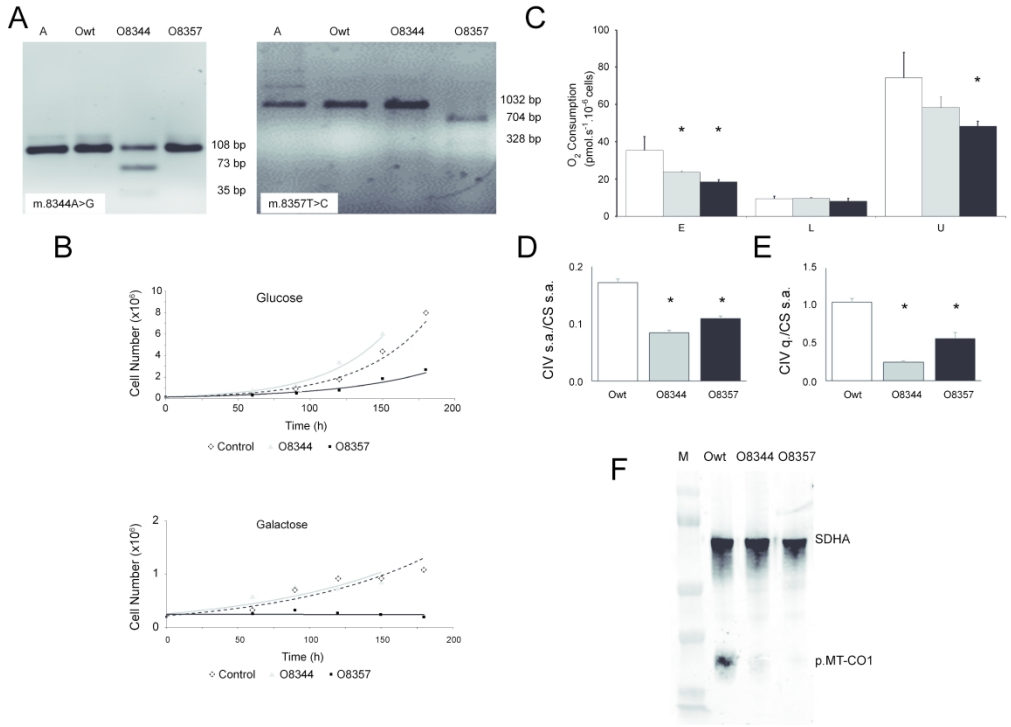
180x109mm (300 x 300 DPI)



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Probands + Relatives	Mutation	References
2 + 1	m.8344A>G (2)	1-4
1 + 1	m.8344A>G (1)	5,6
4	m.8344A>G (3) + MD (1)	7,8
1	MD (1)	9,10
1	m.8344A>G (1)	11
1 + 8	m.8344A>G (1)	12
1	SD (1)	13
1	m.8363G>A (1)	14
1	m.3243A>G (1)	15
1	m.8344A>G (1)	10,16
1	m.3264T>C (1)	17 NCP
1 + 17	m.8344A>G (1)	18
1 + 7	m.8344A>G (1)	19-21
1 + 2	m.8363G>A (1)	22
1 + ?	MD (1)	23
1	m.8344A>G (1)	24
2 + 2	m.8344A>G (2)	25
1	SD (1)	26
1	m.8344A>G (1)	27
3	SD (3)	28
1	m.3271T>C (1)	29 only one small lipoma
2	m.8344A>G (2)	30
2	m.8344A>G (2)	31
1 + 2	m.8363G>A (1)	21,32
1	m.3243A>G (1)	33 Mutation % ≤ 0.102
1	m.8344A>G (1)	21
1	m.8344A>G (1)	34
1	m.8344A>G (1)	35
1	m.8344A>G (1)	36
1	m.4302A>G (1)	37 NCP
1	m.8344A>G (1)	30,38
1 + 6	m.8344A>G (1)	39
4 + 3	m.8344A>G (4)	40
1 + 1	m.8344A>G (1)	41
1	m.8344A>G + m.14484T>C (1)	42
1	m.8344A>G (1)	43
2 + 2	m.8344A>G (1) + m.8363G>A (1)	44
1	m.8344A>G (1)	45
1 + 9	m.8344A>G (1)	46

Supplemental Table. Multiple symmetric lipomatosis and mitochondrial DNA mutations. MD, multiple deletions. SD, single deletion. NCP, Non-confirmed pathogenicity.

19 probands + 3 relatives are reported in [47] but they do not clarify whether some of them have been previously described.

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